

1.0 AMENDMENT

1.1 IN THE SPECIFICATION:

Please amend the following paragraphs to read as follows:

On page 37, at paragraph 2b), line 25 to line 30:

subjecting the extract to fractionation *via* column chromatography, using a series of chromatography columns, such as phosphocellulose (P-11), DEAE cellulose, Phenyl Sepharose™, hydroxylapatite, Mono Q, Mono S™ and the like; and preferably, subjecting the extract to fractionation *via* column chromatography using, in sequence, a phosphocellulose, Phenyl Sepharose™ affinity chromatography medium (GE Healthcare [formerly Amersham Biosciences, Inc.], Waukesha, WI), hydroxylapatite, Mono Q™ and Mono S™ columns (GE Healthcare [formerly Amersham Biosciences, Inc.], Waukesha, WI); and

On page 98, at paragraph 2, line 13 to line 19:

The exemplary purification method of fractionation *via* column chromatography, using a series of chromatography columns, such as phosphocellulose (P-11), DEAE cellulose, Phenyl Sepharose™, hydroxylapatite, Mono Q™, Mono S™ and the like, as disclosed herein represents one method to prepare a substantially purified P-TEFb protein, subunit or peptide. This method is preferred as it results in the substantial purification of the P-TEFb protein, subunit or peptide in yields sufficient for further characterization and use. However, given the DNA and proteins provided by the present invention, any purification method can now be employed.

On page 135, at paragraph 3, line 27 to page 137, line 9:

The DNase inhibitor, an activity necessary for *in vitro* transcription (*Biochimie* 69:1199-1205, 1987), eluted between 0.29 and 0.31 M HGKEDP and P-TEFa eluted between 0.35 and 0.4 M HGKEDP. The 0.3 M HGKEDP step from phosphocellulose was dialyzed to 0.1 M HGKEDP and loaded onto a 100 ml DEAE cellulose column. A gradient elution from 0.1 to 0.5 M HGKEDP was performed. TFIIE eluted at 0.12 to 0.15 M HGKEDP, RNA polymerase II at 0.25 to 0.37 HGKEDP, and factor 2 at 0.18 to 0.22 M HGKEDP. The 0.4 M HGKEDP step from phosphocellulose was concentrated using Centricon-30™ centrifugal filter unit concentrators

(Millipore Corporation, [formerly Amicon] Billerica, MA) and then dialyzed versus 75 mM HGKEDP for 2 h. The 0.75 M HGKEDP step from phosphocellulose was dialyzed to 0.15 M HGKEDP, and passed through a 100 ml DEAE-cellulose column. This DEAE flowthrough was loaded onto an 8 ml FPLC Mono S™ column equilibrated in 0.15 M HGKEDP. The Mono S™ column was subjected to a gradient elution from 0.15 to 0.5 M HGKEDP. P-TEFb eluted between 0.25 and 0.29 M HGKEDP.

On page 137, at paragraph 2, line 19 to page 138, line 3:

Scheme 1: P-TEFb was purified from 90 ml of K_cN (approximately 3.0 g protein). P-TEFb eluted between 0.55 and 0.65 M HGKEDP during gradient elution of a 500 ml P-11 column from 0.15 M to 1.0 M HGKEDP. P-TEFb containing P-11 fractions (250 ml) were adjusted to 0.5 M HGAEDP (HGKEDP with ammonium (NH₄)₂SO₄ substituted for KCl) followed by loading onto a 26 ml Phenyl Sepharose™ column which was eluted with a gradient from 0.5 M to 0 M HGAEDP. P-TEFb eluted from Phenyl Sepharose™ between 0.12 M and 0 M HGAEDP. P-TEFb containing fractions (23 ml) at 0.06 M HGAEDP (equivalent to 150 mM HGKEDP by conductivity) were pooled and allowed to flow through an 8 ml Mono Q™ equilibrated in 0.15 M HGKEDP directly onto a 1 ml Mono S™ column. The Mono S™ column was then eluted with a gradient from 0.15 M to 0.45 M HGKEDP during which P-TEFb eluted in 2.5 ml (50 mg protein) between 0.25 M and 0.29 M HGKEDP. A 200 µl sample from Mono S™ fraction 30 was loaded onto a 4.25 ml, 18%-35% glycerol gradient with a 500 ml 1M HGKE overlay and centrifuged at 55,000 rpm (287,000 g_{av}) in a Beckman SW 55 Ti rotor at 1°C for 44 h.

On page 138, at paragraph 1, line 5 to line 18:

Scheme 2: P-TEFb was purified from 155 ml (265 mg protein) of P11 0.4M to 0.75 M step from embryonic nuclear extract. The initial P-11 step fraction was adjusted to 0.75 M HGAEDP before loading onto a 26 ml Phenyl Sepharose™ which was then gradient eluted from 0.5 M to 0 M HGAEDP. P-TEFb activity eluted between 0.12 M and 0M HGAEDP in 17 ml and was then dialyzed to 175 mM HGKEDP before being passed through a 5.0 ml DE-52

column. The DE-52 flowthrough (19ml, 3.4 mg protein) was loaded directly onto a 1 ml Mono S™ column which was gradient eluted from 175 mM to 500 mM HGKEDP. P-TEFb activity eluting between 0.25 M and 0.29 M HGKEDP was dialyzed and loaded onto a 1 ml Mono Q™ column at 50 mM HGKEDP, followed by gradient elution from 50 mM to 450 mM HGKEDP. P-TEFb activity was found in both the column flowthrough and early gradient fractions. Both pools of P-TEFb activity were combined and rechromatographed over a 1 ml Mono S™ column loaded at 75 mM and step eluted at 400 mM HGKEDP. P-TEFb eluted in two 0.2 ml fractions. A 125 µl sample from one 0.2 ml fraction was loaded onto a 5 ml, 15%-35% glycerol gradient and centrifuged at 55,000 rpm (287,000 g_{av}) in a Beckman SW 55 Ti rotor at 1°C for 40.5 h.

On page 143, at paragraph 3, line 27 to page 144, line 10:

P-TEFb used in FIG. 1 was purified from K_cN as described in Example 1. Phosphocellulose, Phenyl-Sepharose, Mono Q™ and Mono S™ columns were used. A sample of the Mono S™ purified material was analyzed on a glycerol gradient as described in Example 1. The P-TEFb used in FIG. 2A, FIG. 2B and FIG.3 was purified from *Drosophila* embryonic nuclear extract as described in Example 1 with one addition. Material eluting from the Phenyl-Sepharose column was loaded directly onto a 10.0 ml ceramic Hydroxyl-apatite column (Bio-Rad CHT10). The column was then eluted with a linear gradient of potassium phosphate from 10 mM to 750 mM in 25 mM HEPES, pH 7.6, 15% glycerol. P-TEFb eluted between 400 mM and 500 mM phosphate. Pooled fractions containing P-TEFb were then dialyzed and chromatographed on Mono S™. P-TEFb eluting from Mono S™ still had significant nucleic acid contamination so the material was subjected to chromatography on Mono Q™ followed by Mono S™ for re-concentration. The peak fraction from the final Mono S™ column contained about 0.5 mg/ml P-TEFb.

On page 187, at paragraph 2, line 20 to page 188, line 2:

The transformed DE3 cells were grown to OD₆₀₀ = 0.6 and induced with 1 mM IPTG. After a 3 hour induction, the cells were collected and lysed by passing through a French press

three times. The lysate was subjected to centrifugation at $15,000 \times g$ for 30 minutes. The pellet was solubilized in 0.1 M TUS (20 mM Tris, pH7.5, 0.1 M NaCl and 7M Urea) and loaded onto a Mono QTM column (Pharmacia Co.). The flow through (FT) fraction of the Mono QTM column was loaded onto a Mono STM column (Pharmacia Co.). The flow-through fraction of the Mono STM column was subjected to dialysis against Phosphate buffer (20mM phosphate, pH7.0). The dialyzed solution was centrifuged at $15,000 \times g$ for 30 minutes. The pellet was suspended in Phosphate Buffer (20mM phosphate, pH7.0) and used to generate rabbit antibodies following standard protocols (Pocono Rabbit Farm and Laboratory, Inc.). Preimmune serum was obtained before injection of antigen (the human P-TEFb small subunit) into the rabbit. Test bleeding was performed 42 days after the first injection of the antigen and antisera were generated monthly after the test bleeding.